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ANTIMICROBIAL ACTIVITIES OF THREE INDIAN MEDICINAL PLANTS

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Abstract: The antimicrobial activities of root, leaf and stem extracts of three medicinal plants, Barleria prionitis L. (Acanthaceae), Withania somnifera L. Dunal (Solanaceae) and Helianthus annus L. (Compositae) in water, methanol and dichloromethane were evaluated against a few microorganisms (bacteria - Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi and fungi - Aspergillus niger, Aspergillus flavus, Aspergillus awamori, Fusarium oxysporium and Penicillium notatum). Promising antimicrobial potential was observed for the root and leaf extracts of the plants in methanol and dichloromethane. Barleria prionitis root extract in methanol presented highest activity against Bacillus subtilis among all the tested microorganisms. Water extracts, in general showed less antimicrobial activity as compared to other solvents. The antimicrobial activity of the plant extracts was found to be dose dependant and varied with the type and concentration of the extract as well as type of microbial species. The results were compared with the activity of some standard antibiotics.

Key words: Medicinal plants, Antibacterial, Antifungal

INTRODUCTION

Infectious diseases account for high a proportion of health problems particularly in the developing countries. Their treatment is becoming difficult due to increasing resistance in many pathogenic microorganisms to most of the antibiotics, because of their indiscriminate use [1]. This situation has necessitated the search for new antimicrobial substances from various sources such as microorganisms, animals and plants [2,3]. A large number of people in many countries like India and China still use folklore medicinal plants to cure sicknesses. The increasing interest in use of Indian medicinal plants in developing countries has led to intensified efforts in the documentation of ethnomedical data of medicinal plants [4,5] and in understanding their properties, safety and efficiency. It is necessary from the scientific point of view, to establish a rational relationship between chemical, biological and therapeutic activities of folklore medicines [6,7]. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Plant derived drugs serve as a prototype to develop more effective and less toxic medicines. There has been a growing interest to evaluate plants possessing antimicrobial activities for various diseases [8]. Number of studies dealing with antimicrobial screening of medicinal plant extracts are being conducted in different countries [9,10].

As a part of our investigations of local plants for their different biological activities [11-15], we selected three medicinal plants for determining their potential to control a few microbial pathogens. *Barleria prionitis* L. (Acanthaceae), *Withania somnifera* L. Dunal (Solanaceae) *and Helianthus annus* L. (Compositae) are known for their pharmacological properties in the Indian traditional system of medicine

and their varied medicinal significance has been reported. Decoction from *Barleria* is reported to have diaphoretic and expectorant properties and used in dropsy, tooth ache, boils, swelling, asthma and whooping cough. Similarly, *Withania somnifera* is used for treating inflammatory conditions, lesions, ulcers, scabies, fever etc. and *Helianthus annus* finds applications in controlling bronchial, laryngeal and pulmonary infections, dysentery, malarial fever etc. [16]. This paper presents the potential of these three Indian medicinal plants in the management of some human pathogens.

MATERIALS AND METHODS

Microbial samples: The microbial cultures were obtained from culture collection of School of Life Sciences, North Maharashtra Univerisity, Jalgaon. The bacterial cultures used were *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* and fungi were *Aspergillus niger, Aspergillus flavus, Aspergillus awamori, Fusarium oxysporium* and *Penicillium notatum.*

Plant materials and preparation of plant extracts: The plant materials (roots, leaves and seeds) of *B. prionitis, W. somnifera and H. annus* were collected from the campus of North Maharashtra University, Jalgaon and identified by an expert botanist. They were dried in shade for 15 days and ground to fine powder. Powdered plant materials (30g) were soxhlet extracted in water, methanol and dichloromethane (300ml each) for 5 hours at 100, 65 and 40 ^{0}C, respectively. The extracts obtained were dried in vacuum using rotavapor (Buchi, Switzerland) at 100, 65 and 40 ^{0}C and the yield was estimated.

Antimicrobial assay of the plant extracts: The antimicrobial activity of the plant extracts was assayed by antimicrobial susceptibility test. The bacterial and fungal cultures were grown in nutrient broth and Czapek dox broth at 37 ^{0}C and 28 ^{0}C, respectively. 100 µl of 24 h growth of each microorganism, at a concentration of 10^6 cells/ml, was spread on the surface of nutrient agar for bacteria and Czapek dox agar for fungi in Petri plates. Wells of 8 mm diameter were made with a metal borer. 100 µl of each extract at various concentrations in respective solvents was added in the wells. The plates were refrigerated for 2 hours to allow pre-diffusion of the extracts from the wells into the seeded agar layer and then incubated at 37 °C for 24 h for bacteria and 28 ^{0}C for 48 h for fungi. Zones of inhibition were measured in millimeters from the circumference of the wells to the circumference of the inhibition zone. The assays were repeated at least twice with respective solvents serving as controls. The results were compared with some standard antibiotic discs (HiMedia, Mumbai, India).

RESULTS

Yield of the extracts: Amongst different extracts, the leaf extract of *Helianthus* and root extract of *Withania* showed the highest and root and seed extracts of *Barleria* in methanol and DCM gave the lowest yields (Table 1).

Antimicrobial activity of extracts: The antimicrobial activity of plant extracts was found to be dose dependent and varied with the type and concentration of the extract as well as type of microbial species. The extracts showed good antimicrobial activities at 50 mg/ml and 100mg/ml concentrations. Most of the extracts lacked activity at lower concentrations (Tables 2,3).

The methanolic extract of *Withania* exhibited the best antibacterial activity against *S. aureus* followed by root extract of *Barleria* in methanol and DCM, leaf extract of *Helianthus* and Withania in methanol and DCM, respectively. Similarly, methanolic extracts of *Barleria* roots was found to be the most active against *B. subtilis* followed by its root extract in DCM, leaf extract of *Helianthus* and *Withania* in methanol, and methanolic extract of *Helianthus* roots at 100mg/ml (Table 2). On the other hand, *P. aeruginosa* was found to be the most susceptible to methanolic root extract of *Helianthus* followed by leaf extract of *Withania* in DCM, root extract of *Helianthus* and *Barleria* in DCM, *Withania* root extract in DCM, seed extract of *Barleria* in methanol, respectively at 100 mg/ml (Table 2). Of the various extracts tested

Table 1: Yield profile of different plant extracts

Details of Plant		Yield (gm%)		
Plant species	Plant part used	Solvent used for extraction		
		Water	Methanol	Dichloromethane
Barleria prionitis	Roots	2.83	1.66	2.76
	Leaves	5.0	4.0	2.43
	Seeds	3.43	5.66	2.16
Withania somnifera	Roots	15.16	10.36	4.66
	Leaves	10.96	6.0	2.83
	Seeds	7.33	13.73	7.66
Helianthus annus	Roots	3.0	2.7	2.4
	Leaves	16	5.1	4.33
	Seeds	4.7	5.7	5.0

Table 2: Antibacterial activity of plant extracts extracted in various solvents

Bacterial species	Conc. (mg/ml)	Zone of Inhibition (mm)																											
		Barleria prionitis									*Withania somnifera*									*Helianthus annus*									
		Leaves			Seeds			Roots			Leaves			Seeds			Roots			Leaves			Seeds			Roots		
		W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D
Staphylococcus aureus	100	N	11	12	10	15	14	11	38	32	8	40	29	4	5	7	8	10	11	5	36	30	5	7	8	7	18	9
	50	N	8	9	4	8	8	7	32	27	4	34	21	2	3	5	3	5	6	3	31	24	2	3	4	5	12	5
	25	N	N	N	N	N	N	N	25	16	N	25	16	N	N	N	N	N	N	N	24	18	N	N	N	N	10	N
	10	N	N	N	N	N	N	N	17	8	N	17	11	N	N	N	N	N	N	N	16	8	N	N	N	N	2	N
Bacillus subtilis	100	4	6	4	5	7	8	7	42	38	5	28	25	4	7	6	5	7	8	5	31	6	7	9	7	5	24	5
	50	2	3	2	2	2	3	2	38	34	2	22	20	3	4	3	2	4	4	3	27	4	5	6	5	3	18	2
	25	N	N	N	N	N	N	N	31	27	N	19	16	N	N	N	N	N	N	N	20	N	N	N	N	N	12	N
	10	N	N	N	N	N	N	N	23	19	N	16	8	N	N	N	N	N	N	N	12	N	N	N	N	N	8	N
Pseudomonas aeruginosa	100	5	8	9	8	11	7	8	9	11	5	8	14	6	8	7	6	9	11	6	9	5	6	9	10	7	18	13
	50	3	4	5	6	5	4	3	5	6	3	4	10	4	5	4	3	4	5	4	5	3	4	4	5	4	14	10
	25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	10	N
	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	2	N
Escherichia coli	100	5	6	5	6	8	7	6	9	8	7	38	16	8	12	10	6	9	7	7	11	8	21	13	10	7	9	8
	50	2	3	3	3	4	3	4	6	4	3	30	12	6	8	6	4	5	4	5	6	4	16	7	6	4	5	5
	25	N	N	N	N	N	N	N	N	N	N	26	N	N	N	N	N	N	N	N	N	N	13	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	18	N	N	N	N	N	N	N	N	N	N	5	N	N	N	N	N
Salmonella typhi	100	5	6	7	6	8	8	8	28	9	6	31	25	7	8	7	9	11	8	6	10	7	6	9	8	7	11	8
	50	3	4	5	3	4	6	6	21	5	4	28	20	5	6	5	6	6	5	4	7	5	4	5	5	4	6	5
	25	N	N	N	N	N	N	N	15	N	N	22	15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	7	N	N	14	7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

W- Water, M- methanol, D- Dichloromethane N- No antibacterial activity

Table 3: Antifungal activity of plant extracts extracted in various solvents

Fungal species	Conc. (mg/ml)	Zone of inhibition (mm)																											
		Plant species used																											
		Barleria prionitis									*Withania somnifera*									*Hekanthus annus*									
		Leaves			Seeds			Roots			Leaves			Seeds			Roots			Leaves			Seeds			Roots		
		W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D	W	M	D
Aspergillus flavus	100	2	3	3	2	4	3	2	16	9	5	15	12	3	5	6	3	6	4	6	17	8	4	6	4	3	10	4
	50	N	8	9	4	8	8	7	8	6	2	9	9	2	2	2	N	4	3	N	10	6	N	2	3	N	6	2
	25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Aspergillus niger	100	3	4	2	4	5	4	3	18	8	6	16	14	2	6	3	2	6	4	2	15	10	N	7	4	N	12	5
	50	N	N	N	N	N	N	N	10	4	N	6	4	N	2	2	N	3	2	N	7	4	N	2	N	N	3	N
	25	N	N	N	N	N	N	N	4	N	N	N	N	N	N	N	N	N	N	N	2	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Aspergillus awamori	100	2	4	2	3	6	3	2	12	9	N	15	10	N	3	2	N	5	2	3	14	8	2	6	3	N	10	4
	50	N	2	N	N	N	N	N	6	6	N	6	4	N	N	N	N	2	N	N	5	3	N	2	N	N	4	N
	25	N	N	N	N	N	N	N	N	N	N	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fusarium oxysporum	100	N	4	2	N	5	4	N	18	10	N	10	7	N	7	4	N	4	2	N	10	7	N	8	4	N	6	5
	50	N	2	N	N	2	N	N	13	4	N	5	3	N	4	3	N	N	N	N	6	3	N	3	2	N	2	2
	25	N	N	N	N	N	N	N	7	2	N	2	N	N	N	N	N	N	N	N	3	2	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Penicillium notatum	100	N	N	N	N	N	N	N	9	7	N	7	6	N	N	N	N	N	N	N	11	5	N	N	N	N	8	N
	50	N	N	N	N	N	N	N	6	5	N	4	3	N	N	N	N	N	N	N	7	2	N	N	N	N	5	N
	25	N	N	N	N	N	N	N	3	2	N	N	N	N	N	N	N	N	N	N	3	N	N	N	N	N	N	N
	10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	2	N	N	N	N	N	N	N

W- Water, M- methanol, D- Dichloromethane N- No antifungal activity

Table 4: Antimicrobial activity of some standard antibiotics . P = penicillin (10 unit), A = Ampicillin 10µg, C = Cefatoxime-30µg, V = vancomycin -30µg, O = Oflaxacin, T = tetracyclin

Microorganisms	Inhibition zone (mm)					
	P	A	C	V	O	T
Bacterial species						
ıyllococcus aureus	14	13	14	17	27	24
llus subtilis	11	14	10	13	28	22
domonas aeruginosa	10	9	48	10	42	30
erichia coli	14	9	10	22	31	26
onella typhi	17	15	10	20	30	25
al species	Nystatin					
rgillus flavus	13					
rgillus niger	12					
rgillus awamori	11					
rium oxysprum	14					
cillium notatum	20					

against *E. coli*, *Withania* leaf extract in methanol was found to be most active at all concentrations. Some other extracts also did show activity but only at higher concentrations (Table 2). Similarly, *Withania* leaf extract in methanol also showed the most promising antibacterial activity against *S. typhi* followed by root extract of *Barleria* in methanol and leaf extract of *Withania* in DCM (Table 2).

The most promising antifungal activities were shown by methanolic (i) leaf extract of *Helianthus* against *A. flavus*, (ii) root extract of *Barleria* and leaf extract of *Helianthus* against *A. niger*, (iii) leaf extract of *Withania* against *A. awamori*, (iv) root extract of *Barleria*, against *F. oxysporium* and (v) Leaf extract of *Helianthus* against *P. notatum* (Table 3).

Table 4 presents the antimicrobial activity of some standard antibiotics. Oflaxacin showed good overall activity against the bacterial species tested but Cefataxime showed the most promising activity against *P. aeruginosa* (Table 4). Nystatin was found to be active against all fungal species tested with the most promising activity being against *P. notatum* (Table 4).

DISCUSSION

The microorganisms included in this study are involved in various pathological conditions in humans. The gram positive bacterium *S. aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning [17] and is a major cause of hospital acquired infections of surgical wounds and infections associated with indwelling medical devices. It is also known to rapidly develop resistance to many antimicrobial agents [18]. *B. subtilis*, rod shaped aerobic bacterium, is capable of forming resting spores, resistant to extreme environmental conditions and is reported to have pathogenic role [19]. *Pseudomonas* is an aerobic, nonfermentative, oxidase positive bacillus which mainly causes urinary tract infection, wound or burn infection, chronic otitis media, septicemia etc. in humans [20]. *Pseudomonads* are difficult to control by therapeutic means because of their characteristic to develop resistance to most of the drugs used in surgical infections and burns. They are also found to be responsible for infantile diarrhea and sepsis infections [21].

The gram negative bacterium *E. coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia [22,23]. The varieties of *E. coli* that cause diarrhea are classified into named pathotypes, including enterotoxigenic, enteroinvasive, entero-pathogenic, and enterohemorrhagic *E. coli*. Individual strains of each pathotype possess a distinct set of virulence associated characteristics that determine the clinical, pathological and epidemiological features of the diseases they cause [24]. *S. typhimurium* can be found in a broad range of hosts as well as in the environment. Its infection is a serious public health problem in developing countries and represents a constant concern for the food industry. The severity and the outcome of a systemic *Salmonella* infection depend on the virulence of the bacterial strains, the infectious dose as well as the genetic makeup and immunological status of the host [25]. The primary route by which humans acquire infection is by consumption of contaminated food of animal origin. Many species of *Aspergillus* and *Penicillium* are involved in the pathogenesis of Aspergillosis causing health hazards to humans. Some species of *Aspergillus* and *Fusarium* are also responsible for ocular infections [21].

Extracts from three medicinal plants exhibited good antimicrobial activities against most of the microbes tested. Results of methanolic root extract of *Barleria* against *B. subtilis*, methanolic root leaf extract of *Withania* and *Helianthus* against *S. aureus* are particularly promising.. The antifungal activity of these extracts was also encouraging, though was not as strong as antibacterial. The extracts showing encouraging activities were found to exhibit a broad spectrum of action affecting growth of most of the tested organisms. Active constituents responsible for

pharmacological activities of these three medicinal plants have been studied by some of the researchers. β-sitosterol and scutellarein-7-rhamnosylglucosides have been detected in *Barleria*. Several alkaloids particularly, withanoloids, cuscohygrine, anahygrine, tropine, pesudotropin, anaferin, isopelletierine, dulcitol and 3-tropyltigloate have been detected in *W. somnifera*. Particularly, *Withaferin* A has been found to provide many pharmacological activities. Saponins, flavonoids, carotenoids have been detected in *H. annus* [16].

Further investigations are needed to ascertain the observed promising antimicrobial potential of these plants by checking antimicrobial potential of the active constituents and their structure activity relationship as well as toxicity studies by conducting animal trials. Our data show that plant extracts have good potential as antimicrobial compounds and can be used in the treatment of infectious diseases caused by microbes. Even though the concentration of the plant extracts to attain similar kind of antimicrobial activity is higher as compared to the standard antibiotics, they can serve as prototype to develop effective and less toxic alternative medicines. *In vitro* results from our study provide some scientific validation of the widespread use of these plants in traditional medicine.

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